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Abstract: The real time knowledge of dairy milk composition can be used as a tool to guarantee milk quality and safety, offering additional information for dairy producers and consumers. To carry out these in situ analyses, methodologies based on Near Infrared (NIR) portable sensors have a great potential as an advisory tool. The main goals of the present work have been to develop a methodology using a hand-held portable NIR spectrophotometer to collect raw milk spectra, including the development of calibration models for the analysis of protein, fat and solids-non-fat (SNF) of raw milk and further to transfer the developed models to another portable unit. A total of 542 fresh milk samples were scanned over the NIR spectral range (1600-2400nm) using a hand-held MicroPhazirTM (MP) NIR spectrometer and different instrumental configurations. The best results for repeatability and reproducibility calculated as root mean squared (RMS) were obtained using a 17 mm cuvette thickness. The displayed predictive ability of calibration models measured as Standard error of prediction/Standard error of cross validation were 0.96; 0.72 and 0.83 for fat, protein and SNF contents, respectively. For cloning purposes an additional MP unit (satellite) has been used. A standardization set of 10 samples enabled standardization of both instruments. After applying standardization matrix, Standard error of differences between master and satellite reached great reduction, 68% for fat, 66 % for protein and 54 % for SNF. Moreover, the demonstrated ability of sharing calibration models among several units is essential for implementation of portable instruments for in-situ analysis to provide indicators of milk composition at farm level.

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1
2 # Matching portable NIRS instruments for *in situ* monitoring indicators of milk composition

3

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Abstract

The real time knowledge of dairy milk composition can be used as a tool to guarantee milk quality and safety, offering additional information for dairy producers and consumers. To carry out these *in situ* analyses, methodologies based on Near Infrared (NIR) portable sensors have a great potential as an advisory tool. The main goals of the present work have been to develop a methodology using a hand-held portable NIR spectrophotometer to collect raw milk spectra, including the development of calibration models for the analysis of protein, fat and solids-non-fat (SNF) of raw milk and further to transfer the developed models to another portable unit. A total of 542 fresh milk samples were scanned over the NIR spectral range (1600–2400nm) using a hand-held MicroPhazir^{TM} (MP) NIR spectrometer and different instrumental configurations. The best results for repeatability and reproducibility calculated as root mean squared (RMS) were obtained using a 17 mm cuvette thickness. The displayed predictive ability of calibration models measured as Standard error of prediction/Standard error of cross validation were 0.96; 0.72 and 0.83 for fat, protein and SNF contents, respectively. For cloning purposes an additional MP unit (satellite) has been used. A standardization set of 10 samples enabled standardization of both instruments. After applying standardization matrix, Standard error of differences between master and satellite reached great reduction, 68% for fat, 66 % for protein and 54 % for SNF. Moreover, the demonstrated ability of sharing calibration models among several units is essential for implementation of portable instruments for in-situ analysis to provide indicators of milk composition at farm level.

Keywords: *MEMS-NIR, raw milk, in-situ NIRS analysis, standardization, calibration transfer*

69 **Abbreviations**

70 FNS: Foss NIRSytem 6500 monochromator

71 FTIR: Fourier Transform Infrared

72 GH: Global H

73 INIA: National Institute for Agricultural and Food Research

74 MBM: Meat and Bone Meal

75 MEMS: Micro-Electro-Mechanical System

76 MP: Microphazir ™ NIRS Instrument

77 MPLS: Modified Partial Least Square

78 MP-SERIDA: Microphazir ™ NIRS Instrument- Regional Institute for Research and

79 Agro-Food Development

80 MP-UCO: Microphazir ™ NIRS Instrument- University of Cordoba

81 NH: Neighbor Distance

82 NIRS: Near Infrared Spectroscopy

83 PDF: Precision Dairy Feeding

84 PDM: Precision Dairy Management

85 PLF: Precision Livestock Farming

86 R^2_{cv}: Coefficient of Determination in Cross-Validation

87 RMS(C): Root Mean Square of Differences Corrected for the Bias

88 SD: Standard Deviation

89 SECV: Standard Error of Cross-Validation

90 SED: Standard Error of Difference

91 SEP: Standard Error of Prediction

92 SNF: Solids-Non-Fat

93 SNV: Standard Normal Variate

94 SNVD: Standard Normal Variate plus Detrend

95 st1: Cloning set comprising 1 sample (the sample closest to the center of the population)

96 st10: Cloning set comprising 10 samples

97 TMR: Total Mixed Ration

98 UCO: University of Cordoba

99

100 **1. Introduction**
101
102 In the near future more and more dairy farms will uptake sophisticated Precision
103 Livestock Farming (PLF) by sensors systems to support farm management. PLF is a
104 combination of developing animal sensing (sensors) tools and decision-making process
105 at the farm level. These precision systems include an instantaneous knowledge of dairy
106 milk composition; this information can be used as a tool to guarantee milk quality and
107 safety. It also has the potential to support animal feed suppliers, human-food retailers
108 and other players along the supply chain to make better choices. The current challenge
109 for PLF is the integration of the technology in the farm but not only to the pioneering
110 farms (Halachmi, 2015). Banhazi, Babinszky, Halas & Tscharke (2012) outlined the
111 potential role that PLF can play in ensuring that the best possible management processes
112 are implemented on livestock farms increasing farm profitability and quality of milk
113 products for consumers.
114 A new, alternative model for labour-efficient dairy production is emerging. Part of this
115 trend in automation, robotic milking - an example of "precision dairy management"
116 (PDM) - reduces labour requirements and minimize food safety risks (Rodenburg, 2012;
117 Bewley, Russell, Dolecheck, Borchers, Stone & Wadsworth, 2015). However, in order
118 to fully exploit the potential of this changing trend in dairy management, specific
119 technologies should be considered together with the most widespread as, electronic
120 radio frequency identification systems, robotic milking and calf- feeding systems,
121 cameras, microphones, etc. These technologies allow control with precision as feed
122 quality as the final product, milk, which could include under the term of Precision Dairy
123 Feeding, (PDF). Taking into account that feed cost represents the most significant item
124 of the total costs in milk production, and that in recent years, the volatility of the prices
125 of cereals and flour protein, has been recurrent in world markets, it makes necessary to

126 use alternative rations, as far as possible, trying to introduce raw materials of low cost,
127 and the greatest possible use of local resources and by-products, often based on a total
128 mixed ration (TMR) that combines all ration ingredients into a single feed mix. This
129 complicates the nutritionist roles, who must formulate rations with many raw materials,
130 even with nutritional value and composition little known to them, maintaining quality
131 and assessing milk safety. This situation of fragility of the dairy sector at the global
132 level is causing, innovative nutritionists to look for alternatives such as NIRS
133 instruments to be used as a necessary tool in PDF. There are numerous works in the
134 NIR literature applying NIRS technology to milk analysis (reviewed by Holroyd, 2013).
135 They have shown that it is possible to obtain high or moderate accuracy and precision in
136 calibration models to predict the main chemical constituents. Papers dealing with the
137 application of NIR to liquid milk can be split into several areas that involve; the
138 determination of milk composition, authentication of cow feeding regimes and
139 geographic origin of milk, including milk classification, calibration robustness,
140 industrial applications and the measurement of milk microbiological content.
141 A high percentage of water content in samples to analyze could interfere with NIRS
142 analyses. Water content in fresh milk is one of the major contributors to the variation in
143 the NIR spectra due to the strong absorption bands of O-H groups in the NIR region,
144 which can create a critical interference in quantitative analysis. Most of the research
145 milk works are carried out using homogenized and dried samples (DESIR method)
146 (Núñez-Sánchez et al., 2016).
147 The use of NIRS technology on-farm, for the analysis of forage and TMR has been
148 demonstrated scientifically and there are some commercial solutions developed, such as
149 a NIR Analyzer installed directly on the self-propelled mixer wagon or in the shovel of
150 the front loader. It is able to predict dry matter for each ingredient during the loading

151 phase recalculating automatically the quantity to load to maintain a consistent ration
152 (https://www.dinamicagenerale.com/Media/Default/Catalogues/PrecisionFeeding-ENG-
153 LOW.pdf, 2016). However, research about the employment of portable NIRS sensors,
154 susceptible to use for the on-site control of milk obtaining information on individual
155 cow state is very limited or almost non-existent (Kawasaki et al., 2008; dos Santos,
156 Lopo, Páscoa & Lopes, 2013). Therefore, it is urgent and important, to get scientific
157 information about the potential of portable NIRS instruments for the analysis of raw
158 milk, existing currently in the market.
159 The challenge facing this applied research is that the instruments more consolidated in
160 the market, are not designed for this specific purpose of analyzing complex liquids such
161 as milk. In terms of spectral characteristics and physico-chemical properties, it is
162 necessary to show their adaptation and feasibility for the analysis of quality of raw milk.
163 The main goals of the present work are to develop a new methodology based on use of
164 hand-held portable NIR spectrophotometer for the analysis of fat, protein and solids-
165 non-fat (SNF) in raw milk. Further we will evaluate the transferability of the developed
166 methodology and calibration models to a second portable NIRS unit. Finally we will
167 study the alternative of sharing prediction models among several units as essential tool
168 for implementation of portable NIR instruments for in-situ analysis to provide indicators
169 of milk composition at farm level.
170

171 **2. Material and methods**
172
173 *2.1. NIR instruments and analysis methods*

174 - 1) A Foss NIRSystem 6500 monochromator (FNS). This is an at-lab instrument,
175 working in a wavelength range between 400 and 2500 nm, equipped with
176 transport module under controlled environmental conditions (temperature 24ºC

177 ± 1ºC, relative humidity 50% ± 10%). This instrument was used as a qualitative
178 reference instrument to optimize sampling strategy and to evaluate the loss of
179 spectra performance using portable instrument with small scanning window and
180 narrow wavelength range. Spectra were collected using a liquid opaque quartz
181 cuvette, reusable, with a 17 mm pathlength (C17) and an aluminum backside
182 (FOSS. Ref US-ISIH-0398) for trans-reflectance measurements, combining
183 reflectance and transmittance together into a single mode. The spectra data were
184 recorded in reflectance mode (log 1/R) with ISI scan software (Infrasoft
185 International Inc., Port Matilda, PA, USA). Each sample was analyzed in
186 duplicate and each spectrum was the average of 32 scans performed on liquid
187 milk.

188 - 2) MicroPHAZIR ^{TM} (MP) from Thermo Scientific, with a scanning window of 4
189 mm diameter (sampling area of 0.13 cm^2). All diffuse reflectance spectra were
190 computed in a wavelength range between 1600 and 2400 nm, with a non-constant
191 interval of around 8 nm (pixel resolution 8 nm, optical resolution 12 nm) using a
192 hand-held micro-electro-mechanical system (MEMS) digital transform as portable
193 NIRS sensor. The instrumental conditions to collect raw milk spectra with this
194 portable NIR were optimized modifying the parameters:

195 a) Sample presentation - two cuvettes have been assayed; the first one was C1 quartz
196 cuvette, with a 1 mm pathlength and reusable. A liquid analysis adapter, to avoid
197 NIR radiation losses through the quartz backside, was coupled to MP for the
198 analysis of milk samples with this cuvette. The second one was the C17 quartz
199 cuvette with an aluminum backside, described above (Foss NIRSystem 6500).

200 b) Number of scans to average for collecting one spectrum - the range evaluated was
201 between 5, 10 and 80 scans/spectra. Five is the minimum value to be recorded using

202 Phazir Data Management System software (Polychromix, Inc., Wilmington, MA,
203 USA) and 80 is the maximum value.
204 c) Internal reference or external reference for scanning background.
205 For cloning purposes two different units of MP have been used: SERIDA (MP-
206 SERIDA; master instrument) and UCO (MP-UCO; satellite instrument) hand-held
207 NIRS.
208 Nowadays there are other handhelds devices in market, however MP instruments have
209 been selected to develop this research work because being handhelds NIRS they are
210 easy to manage, and only these instruments were available in UCO and SERIDA labs
211 (Modroño, Soldado, Martínez-Fernández & de la Roza-Delgado, 2017).
212
213 *2.2. Samples and pretreatment*
214
215 A total of 552 fresh milk samples were collected between 2014 and 2016 from
216 individual Holstein–Friesian dairy cows of the experimental farm located in the
217 Regional Institute for Research and Agro-Food Development (SERIDA) under different
218 feeding experiments, and from different farms located in the North of Spain (Asturias,
219 Spain), as suppliers from commercial milks looking at variability in their composition
220 through the effect of supplementation, pasture biodiversity, fed different preserved
221 forages (hay and/or silages) or changeability of TMR. Milk samples from experimental
222 cows of SERIDA were taken from each individual animal by using the automatic
223 sampler of Automatic milking system (DeLaval, Spain) and in farms by the farmer.
224 The first 50 fresh milk samples (Set 1) were employed to optimize instrumental
225 conditions, and establish a sampling methodology for obtaining high quality milk NIR
226 spectra using MP-SERIDA spectrophotometer. NIR analyses for this Set 1 were carried
227 out simultaneously on portable MP-SERIDA and FNS as reference at-line instrument.

228 Set 2 comprising 492 milk samples was divided in two different groups selected with a
229 view to covering the whole range of spectral variability and product absorbance values,
230 using the SELECT algorithm included in the WinISI II version 1.50 software package
231 (Infrasoft International, Port Matilda, PA, USA):

232 Group 1 comprising 444 milk samples analyzed in hand-held MP-SERIDA. It was used
233 to develop the calibration models. NIR analyses for this Group 1 were carried out with
234 portable MP-SERIDA.

235 Group 2 comprising 48 milk samples scanned simultaneously on both hand-held
236 instruments, the master MP-SERIDA and in a second MP-UCO unit. This group was
237 divided in two different sub-groups. One sub-group comprising 10 milk samples
238 selected to obtain standardization matrixes and the other one comprising 38 milk
239 samples to validate the transference procedure.

240 As final step for practical performance, 10 milk samples coming from dairy cows of the
241 experimental farm of SERIDA were analyzed using MP-UCO device, to evaluate
242 sample by sample the calibration transfer procedure.

243 All samples were scanned without pretreatment after homogenization by hand mixing
244 for 20-30 sec. The same portion of the sample used to collect spectra in MP instruments
245 was used for reference data analysis (fat, protein and SNF). Reference analyses were
246 carried out using FTIR MilkoScan™ (Foss Electric, Hillerod, Denmark) in the
247 Professional Milk and Agro-food Laboratory of Asturias. This laboratory is accredited
248 under UNE-EN ISO/IEC 17025: 2005 (246/LE476).

249

250 *2.3. Spectral Data and Cloning Processing*

251

252 The first step when starting this research work was to export into *csv format all
253 spectral data collected from MP instruments. After that, the spectral data were adjusted

254 using an interpolation function to get data with a constant step of 2 nm and preserving
255 the shape by interpolation (Fernández Pierna, Vermeulen, Lecler, Baeten, & Dardenne,
256 2010). This adjustment is necessary because the MP spectrometer works in the range of
257 1600 to 2400 nm with a non-constant step.
258 The WinISI software package v. 1.50 (Infrasoft 165 International, Port Matilda, PA,
259 USA) was used to compare FNS *vs* MP spectral data and for chemometric development
260 of MP calibration models. The equations were developed using Modified Partial Least
261 Square (MPLS) as regression method and cross-validation to select the optimal number
262 of factors to avoid overfitting (Shenk & Westerhaus, 1995). Chemical outliers were
263 detected using the Student T test, to check differences between reference and predicted
264 values; samples with a T value of over 2.5 were considered outliers (Mark & Workman,
265 1991).
266 Combined standard normal variate (SNV) plus detrend treatments were used for scatter
267 correction (Barnes & Dhanoa, 1989). First- and second-derivative treatments were
268 tested: 1.4.4.1; 1.8.8.1; 1.10.5.1, and 2.5.5.1, where the first digit is the number of the
269 derivative, the second is the gap over which the derivative is calculated (expressed in
270 data points), the third is the number of data points in a running average or smoothing,
271 and the fourth is the second smoothing (ISI software, 2000).
272 The best fitting equations, selected by statistical criteria for each parameter, on base of
273 the lowest standard error of cross-validation (SECV), highest coefficient of
274 determination in cross-validation (r^2_{cv}) (Williams, 2001; Pérez-Marín et al., 2008;
275 Soldado, Fearn, Martínez-Fernández & de la Roza-Delgado, 2013) and lowest relation
276 value between standard error of prediction (SEP, statistical parameter for testing
277 external validation of the calibration model on 38 milk samples of group 2) and SECV
278 (SEP/SECV) (Savenije, Geesink, van der Palen & Hemke, 2006).

279 Analytical features of NIR developed methodology was compared with reference
280 methods performance on the basis of their laboratory error and were calculated as
281 intermediate reproducibility according to ISO 5725 (ISO5725-1, 1994; ISO 5725-2,
282 1994) definitions: (i) repeatability, indicates the variability observed within a
283 laboratory, over a short time, using a single operator, item of equipment etc., and (ii)
284 intermediate reproducibility (standard deviation SD), intermediate precision relates to
285 the variation in results observed when one or more factors, such as time, equipment and
286 operator, are varied within a laboratory) on 10 different samples of Set 2 and was
287 calculated attending Eq. [1]:

288 $$R = S_R 2\sqrt{2} \quad [1]$$

289 A key factor in the cloning process is the number of samples used both when selecting a
290 procedure for standardizing NIR instruments and when selecting a cloning algorithm
291 (Zamora-Rojas et al., 2012; Pérez-Marín, Garrido-Varo ¬ Guerrero-Ginel, 2006). Since
292 cloning using numerous samples is a more complex procedure, it is advisable to
293 minimize the number of samples to be analyzed in parallel on the two instruments to
294 develop the algorithm. Two strategies using different number of samples were tested: (i)
295 10 samples comprising the cloning set (st10); and (ii) the sample closest to the center of
296 the population (st1). The cloning algorithm used for standardization process was the
297 patented algorithm by Shenk & Westerhaus (2008).
298 The statistic root mean square error (RMS) was used to select and to compare spectra
299 between subsamples in order to determine differences in repeatability and
300 reproducibility conditions (ISO5725-1 & 2, 1994).
301 This statistical parameter as the averaged root mean square of differences corrected for
302 the bias (RMS(c)) between two spectra was calculated using the CONTRAST algorithm

303 included in the WINISI software package, version 1.50 (Infrasoft International, Port

304 Matilda, PA, USA), and the formula to calculate the RMS(c) is Eq. [2]:

305
$$RMS(c) = 10^6 \times \sqrt{\frac{\sum_{i=1}^{n}(y_{im}-y_{ik})^2 - \frac{\left(\sum_{i=1}^{n}(y_{im}-y_{ik})\right)^2}{n}}{n-1}} \quad [2]$$

306 Where;

307 Y_{im} = log (1/R) value of m subsample at a wavelength i (λ_i).

308 $\overline{Y_{ik}}$ = log (1/R) value of k subsample at a wavelength i (λ_i).

309 n = number of wavelengths

310

311 Sample scanning modes giving spectra with the minimum value of RMS was selected

312 for further development of calibration to predict quality parameters in milk. Besides, to

313 evaluate the standardization process, spectra of master and host instrument were

314 compared using the statistic RMS(c).

315 To evaluate the transference process of predictive NIRS models, were selected the

316 Mahalanobis H. Values were calculated for the statistics global H (GH), i.e. the distance

317 of a given sample from the center of the population, and neighbor (NH), i.e. the distance

318 of that sample from its nearest neighbors (Zamora-Rojas et al., 2012) for spectral

319 comparison, and the ratio SEPstandardized / SEPmaster and SEDstandardized /

320 SEDmaster (SED: standard error of difference), to evaluate the transferred models.

321

322

323 **3. Results and discussion**

324

325 *3.1. Sample presentation and NIRS analysis optimization*

326 Prior to statistical assessment it was necessary to optimize sampling strategy to remove

327 those spectra showing low quality. To attempt this work, during this optimization

328 process all spectra were collected with FNS and MP devices. FNS analyzing with C17

329 cuvette was selected as reference instrument for qualitative comparison. To optimize

330 experimental conditions on MP-SERIDA (type of cuvettes, different number of spectra
331 to average and the use of standard or internal reference material) was carried out the
332 comparison between FNS and MP-SERIDA spectra shape.
333

334 The optimization results of spectra collection are shown in Fig. 1. As can be seen the
335 strong absorption of water bands and the small scanning window of MP analyzer make
336 it difficult to obtain spectra comparable to those obtained with the reference instrument.
337 As it is well known, milk is a very complex matrix for NIR analysis, consisting of
338 proteins in colloidal dispersion, fat in emulsion and minerals in solution (Marinori,
339 Monti, Barzaghi & de la Roza-Delgado, 2013). One of the complexities facing us in the
340 analysis of raw milk is the heterogeneity of the sample and its high water content
341 (Schmilovivh, Shmulevich, Notea & Maltz, 2000; Tsenkova et al., 2000). It is an
342 opaque liquid with highly light scattering effect caused by milk fat globules and casein
343 micelles in suspension (Holroyd, 2013). Water content in raw milk is one of the major
344 contributors to the variation in the NIR spectra, due to the strong absorption bands of O-
345 H groups in NIR region, with a basic characteristic region at 1940 nm (Shenk,
346 Workman & Westerhaus, 1992) that could limit the detection of analytes.
347 As can be seen in Fig. 1, the strong NIR absorption bands attributed to water due to the
348 hydrogen bonds have led a high value for log (1/R) around 1940nm (water band),
349 representing the O–H second overtone bending (Williams & Norris, 2001) and a high
350 spectral noise at the end of scanning range when NIR analyses using MP instrument
351 were made with 5 scans to average/sample employing both cuvettes, being much higher
352 noise when the analysis are made with the cuvette C1 plus liquid adapter.
353 On the other hand, the recognition of absorption bands attributed to the other
354 components such as fat or crude protein also was possible related with 2310 and 2180

355 nm, respectively, although they were very weak in comparison with the O-H bands and
356 were more difficult to observe.
357 The following step was to optimize the number of scans to average for collecting one
358 spectrum in MP instrument. To minimize spectra noise different numbers of scans were
359 assayed 5, 10 and 80 scans/spectra. Results have shown that the spectral noise at the end
360 of scanning range was reduced averaging 80 scans/sample and spectra were collected
361 with high sensitivity. This value was selected for further work.
362 Afterwards the use of internal or external reference (material) was optimized. The use of
363 the reference in NIR analysis is necessary to collect background, because all
364 measurements are referred to the background. No differences were observed when
365 analyzing milk samples using external or internal reference. For simplicity the internal
366 reference was selected to collect spectra. This analyze mode avoids carry out and
367 employ an external reference at farm level in order to simplify the analysis.
368 Table 1 shows the results of spectra repeatability and reproducibility for both cuvettes
369 with the statistic RMS using milk samples from Set 1 to compare portable spectra (80
370 spectra to average and internal reference) with those recorded on FNS reference
371 instrument. As can be seen, the best results were obtained using the C17 cuvette with an
372 aluminum backside. Values for FNS (at-lab) were lower than MP being the ratio
373 between at-lab and handheld device 0.5 in repeatability and 0.8 in intermediate
374 reproducibility using C17 cuvette. Selected experimental conditions were: sampling
375 with cuvette C17 and 80 scans/sample to average using the internal reference material.
376 After finishing the optimization procedure to collect spectra using the MP NIRS the
377 samples were scanned using MP instrument to develop calibration models.
378
379 *3.2. Calibration models*

380 Calibration (Group 1) and validation (Sub-group 2) sets descriptive statistics (range,
381 mean and standard deviation) are shown in Table 2. For each parameter, the validation
382 set comprised samples representative of the total variance, all values lying within the
383 range established for the calibration set. Both sets displayed, for range values, ratios
384 calibration/validation from 0.88 to 1.28 and similar values for mean, and standard
385 deviation (SD). As can be seen the average values of fat, protein and SNF percentage
386 are similar to those established for milk quality payment. However, a high variability is
387 observed in both populations, samples with high levels of fat and protein, and others
388 with very low levels. Related with reference method error, the values were 0.114 % for
389 fat; 0.063 for protein and 0.128 for SNF.
390 After assaying different derivative mathematical treatments to develop NIR calibrations
391 (see Material and Methods section). The best results were obtained applying SNVD for
392 scatter correction and 1,10,5,1 or 2,6,4,1 as math treatments. These pretreatments
393 yielded the lowest SECV and highest r^2_{cv}. The external validation results were evaluated
394 according to the minimum relation value between SEP/SECV. In base of these statistics
395 finally were select 1,10,5,1 as math treatment for protein content and 2,6,4,1, for fat and
396 SNF. Characteristics of the predictive models are given in Table 3.
397 The cross-validation statistics of calibration models displayed great predictive ability
398 with SECV of 0.102 and r^2_{CV} of 0.961 for fat milk content. For protein content the
399 model selected may be considered good (R^2 =0.758; r^2_{cv} = 0.676; SECV= 0.124%)
400 whilst the model obtained for SNF would enable values for milk to be classified as high,
401 medium or low concentration (R^2 = 0.612; SECV= 0.225%), following Williams'
402 recommendations (2001).
403

The ratio SEP/SECV varied between 0.89 and 1.24. Assuming the SEP is approximately equal to SECV, this ratio is very acceptable with regard to the accuracy of the calibration. (Savenije, Geesink, van der Palen & Hemke, 2006).

Different research works using NIR laboratory instruments have established the usefulness of NIRS technology to predict milk composition and microbiological parameters (Holroyd, 2013). However, it is necessary taking into account that these evaluations were conducted using NIR instruments with wide spectral range and different possibilities of sample preparation and presentation.

In this sense, Tsenkova and co-workers (2000) evaluated the potential of NIRS to measure fat, total protein, and lactose contents of unhomogenized milk for use in dairy management, as a new tool for on-line milk analysis in the process of milking, working in the wavelength range from 400 to 2500 nm with sample thicknesses of 1 mm, 4 mm, and 10 mm based on log (1/T) data. Their found that the accuracy of fat and protein content determination of bovine milk depended strongly on the spectral regions and path lengths and the best results were obtained for the region from 1100 to 2400 nm with 1-mm sample thickness. The SECV for the model based on the first derivative spectral data transformation was 0.110 and the r^2_{cv} was 0.998 for fat content and SECV= 0.096 and r^2_{cv} = 0.848 for protein. With regard to fat content our results shown in Table 3 generally agreed with those reported by these authors by using a portable instrument with a narrow spectral range.

Related with on-line NIR analysis a publication by Masataka and co-workers (2008) provide NIR spectra of raw milk obtained in an automatic milking system (milking robot system) over a wavelength range of 600 nm to 1050 nm (transmittance). The SEP of the validation set for fat was 0.25%, this SEP value represent 200% of SEP reported here (SEP= 0.126). The value of SEP for protein obtained for these authors was 0.15%,

429 again the SEP value obtained in this work for this parameter is slightly lower (SEP =
430 0.124%).
431 Related with the results obtained using portable analyzer designed and developed for
432 raw milk quality analysis during the material purchase in dairy plants (Feng et al., 2013)
433 calibration model shows worse SEP values (0.172 and 0.201 for fat and protein content)
434 than those obtained in this work.
435
436 *3.3. Standardization process*
437 Two standardization matrixes were developed using one milk sample (st1) or 10
438 samples (st10). To evaluate the success of the standardization procedure the first step
439 was focused on the reduction of GH and NH values, in validation set (N=38) (see Table
440 4). These GH values were 1.497 for MP-SERIDA, 20.000 for MP-UCO before
441 standardization and 1.550 after applying standardization matrix developed with one
442 sample (MP-UCOst1). Related with NH the values obtained were 0.858 for MP-
443 SERIDA, and decreasing from 15.309 to 1.043 for MP-UCO after applying
444 standardization matrix. The GH and NH values obtained for MP-UCO before
445 standardization, confirm the need for this process. GH and NH statistics show and
446 excellent agreement between spectra collected in both instruments even when applying
447 only one standardization sample and confirm that standardization successfully reduced
448 spectral differences between both instruments for the validation-test set.
449 Related with the comparison between the spectra recorded in both MP evaluated
450 instruments attending RMS(c) statistic, the best results, those with minor RMS(c), were
451 obtained with the standardization matrix built with 10 samples. The RMS(c) values
452 between master unit and secondary device spectra decreased from 54,590 prior to

453 standardization to 16,493 and 11,818 when applying st1 or st10 standardization
454 matrixes.
455 Fig. 2A and 2B show the mean spectra for the external validation set collected with both
456 handheld NIRS instruments before and after standardization process as raw log (1/R)
457 spectra (A) and after applying first derivative and SNVD mathematical treatments to the
458 spectral data (B). In this Fig. 2 can be seen differences between the spectra before
459 standardization in the 1880–2100 nm range. These log1/R differences are related to the
460 differences between instruments that are the same model device but they are not cloned
461 instruments. Both MP units can vary in photometric response; this is due to detectors,
462 light sources and changes over in the instrumental response function (ageing of sources,
463 replacement of some parts, etc.). However, these spectra differences must disappear
464 after standardization process showing a successful result of the standardization
465 approach.
466 The last step in the calibration transference process was to validate the transferred
467 equations with the external set of samples (Sub-group 2, N=38). Results for external
468 validation on both instruments are shown in Table 5. When the equations were applied
469 to non- standardized spectra from MP-UCO, there was a loss of performance with SEP
470 values of 0.147; 0.810 and 1.663 % for fat, protein and SNF content, respectively.
471 Nevertheless, after applying st1 or st10 standardization matrices SEP from MP-UCO
472 decreased approximately 80 % for protein and 85% for SNF content. Related with milk
473 fat content the standardization process has not too much influence over the reduction of
474 SEP values. Probably, the specific NIRS bands related with fat from 2150 to 2300 nm
475 are not affected by the standardization, because the great differences between the
476 spectra recorded in MP-SERIDA *vs* MP-UCO before standardization are in the 1880–

477 2100 nm range, directly related with protein wavelength ranges (Osborne & Fearn,
478 1986).
479 Additionally, to check the performance of transferred models was calculated the SED,
480 expressed as a difference between NIR analyses on MP-SERIDA and MP-UCO
481 instruments (see Table 5). After applying standardization matrices, SED values between
482 MP-SERIDA and MP-UCO decreased at least eight times for SNF and five times for
483 protein compared to non-standardized results. For fat the reduction was only 1.2 times
484 lower. These SED values were close to SEP values.
485 To include a practical performance, after comparing the standardization procedure
486 between NIR instruments (MP-SERIDA and MP-UCO), 10 milk samples coming from
487 dairy cows of the experimental farm of SERIDA were analyzed with the MP-UCO
488 device and applying both standardization matrices. Results are detailed in Table 6. As
489 can be seen differences between reference and predicted values decrease after
490 standardization. However, we must remark that there are not differences between both
491 standardization matrices. For protein and SNF there are two samples with errors lower
492 using st1 than using st10 standardization matrices. For fat, the prediction of 4 samples is
493 more exact when applying st1. Nevertheless, st10 always has minor sum of residual
494 values than st1.
495 To the best of our knowledge this is the first time that the ability of the
496 MicroPHAZIR^{TM} to predict the milk composition changes of individual cows has been
497 demonstrated. Furthermore, the ability of sharing calibration data among several units is
498 a key point with a great importance for implementation of portable instruments at farm
499 level for *in situ* quality control of milk.
500

501 **4. Conclusions**
502

503 After evaluating different sampling strategies to analyze raw milk samples using the
504 handheld instrument Microphazir^{TM} we can conclude that to obtain satisfactory results it
505 is necessary to average 80 scans to collect one sample spectra using 17mm sample
506 thickness cuvette with an aluminum backside.

507 This study has established a promising ability of this handheld NIR instruments to
508 estimate the individual dairy milk composition changes. Moreover, the calibration
509 models developed showed that the accuracy and precision of the equations obtained
510 using the handheld instrument were similar, in terms of both calibration and validation,
511 to those of the equations obtained on lab based instruments.

512 The promising results for the ability of sharing calibration data (transference procedure)
513 after applying a simple standardization algorithm for spectral adjustment minimized
514 spectral differences between hand-held MicroPhazir analyzers even developed with
515 only one sample have great importance for implementation of portable instruments as a
516 tool for *in situ* monitoring indicators of milk composition.

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518

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523

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634

635 **TABLES**

636 **Table 1.** Repeatability and reproducibility root mean square (RMS) for 80 scans by

637 spectra with C1 and C17 cuvettes types

638

639

Intrument	Cuvette type	Repeatibilty RMS	Reproducibility RMS
MP-SERIDA	C1 (1-mm + adapter)	11190	45270
	C17 (aluminum 17 mm)	5309	4799
FNS	C17 (aluminum 17 mm)	2568	3823

640

641 *MP: MicroPHAZIR NIR instrument*

642

643 **Table 2.** Statistic descriptive values for milk samples in calibration and external

644 validation sets

645

	CALIBRATION (N=444)			**EXTERNAL VALIDATION (N=38)**		
Parameter (%)	**Range**	**Mean**	**SD**	**Range**	**Mean**	**SD**
Fat	2.38 – 6.36	3.67	0.575	2.71 – 4.97	3.57	0.476
Protein	2.58 – 4.00	3.18	0.262	2.47 – 3.37	2.98	0.193
SNF	7.14-9.85	8.73	0.325	7.66-9.12	8.62	0.287

646

647 *SD: standard deviation variation, SNF: solids-non-fat*

648

649 **Table 3**. Statistics for calibrations models developed in MP-SERIDA Master Unit

650

Parameter (%)	SEC	R^2	SECV	r^2_{cv}	SEP
Fat	0.089	0.971	0.102	0.961	0.126
Protein	0.120	0.758	0.139	0.676	0.124
SNF	0.185	0.612	0.225	0.476	0.221

651

652 *SNF: solids-non-fat; SEC: Standard Error of Calibration; R^2: Determination Coefficient of*
653 *Calibration; SECV: Standard Error of Cross-validation; r^2_{cv}: Determination Coefficient of*
654 *Cross-Validation; SEP: Standard Error of Prediction*

655

656 **Table 4**. GH, NH and RMS(c) values for the "cloning set" (N=38) analyzed on the
657 MP-SERIDA and MP-UCO before and after standardization using two matrixes (st1
658 and st10).

659

Parameter	MP-SERIDA	MP-UCO before	MP-UCOst1 after	MP-UCOst10 after
Mean GH	1.497	20.000	1.550	1.839
Mean NH	0.858	15.309	1.043	1.218
RMS(C) (μlog (1/R))	12,965	54,590	16,493	11,818

660 *st1= Sample closest to center of population; st10= 10 samples.*

Table 5. Standard errors of prediction and standard errors of difference in the validation set (N = 38) for the calibrations obtained in the MP-SERIDA and MP-UCO for predicting fat, protein and SNF content in raw milk.

Parameter	SEP				SED		
	MP-SERIDA	**MP-UCO before**	**MP-UCOst1 after**	**MP-UCOst10 after**	**MP-SERIDA *vs* MP-UCO**	**MP-SERIDA *vs* MP-UCOst1**	**MP-SERIDA *vs* MP-UCOst10**
Fat	0.126	0.147	0.167	0.145	0.179	0.193	0.146
Protein	0.124	0.810	0.190	0.178	0.762	0.133	0.179
SNF	0.221	1.663	0.460	0.274	1.573	0.361	0.214

SNF: solids-non-fat

Table 6. Practical performance using calibration models before and after transference procedure, for predicting fat, protein and SNF content in raw milk (N=10).

	Fat				Protein				SNF			
Sample	Ref.	MP-UCO before	MP-UCOst1 after	MP-UCOst10 after	Ref.	MP-UCO before	MP-UCOst1 after	MP-UCOst10 after	Ref.	MP-UCO before	MP-UCOst1 after	MP-UCOst10 after
1	3.39	3.30	3.32	3.43	2.94	1.69	2.81	2.85	8.52	7.16	8.39	8.45
2	3.40	3.32	3.34	3.48	2.77	1.98	3.10	3.15	8.54	7.15	8.41	8.55
3	3.21	3.35	3.36	3.18	2.81	0.85	2.30	2.76	8.24	6.52	8.06	8.52
4	3.33	3.29	3.31	3.36	2.47	1.48	2.72	3.05	7.66	6.82	8.19	8.55
5	3.84	3.72	3.68	3.84	3.20	2.06	3.09	3.12	8.86	7.30	8.46	8.55
6	3.93	3.71	3.67	3.84	2.97	1.68	2.78	2.83	8.45	7.16	8.35	8.44
7	3.86	3.68	3.65	3.80	2.92	1.43	2.62	2.73	8.45	7.06	8.32	8.48
8	3.52	3.39	3.40	3.50	3.06	1.79	2.92	2.98	8.69	7.16	8.40	8.47
9	3.54	3.78	3.72	3.63	3.12	1.86	3.06	3.31	9.03	7.11	8.46	8.72
10	4.38	4.24	4.13	4.34	2.86	1.93	2.96	2.98	8.57	7.50	8.61	8.65

Ref. : Reference data, SNF: solids-non-fat

1 **Figure captions**

2 Fig. 1. Mean spectra of milk (N=25 of Set 1) analyzed averaging 5 scans/sample in MP-
3 SERIDA and FNS instruments and different cuvettes.

4 A) MP-SERIDA: C1 cuvette + adapter module; B) MP-SERIDA: C17 cuvette; C) FNS:
5 C17 cuvette

6

7 Fig.2. Mean spectra for the external validate transfer set (Set 2 (Group 2), N=38
8 samples and 80 scans/spectra) with both instruments. (A) Raw log (1/R) spectra with no
9 pretreatment and (B) First derivative spectra with SNVD treatment. In both plots the
10 line with circles (a) is the MP-SERIDA, (b) the grey solid line is the MP-UCO before
11 standardization and (c) the thick solid black line is the MP-UCO after standardization.

Highlights

- Establishing strategies for on-site milk quality control
- Monitoring in real-time, in-situ and without pre-treatment milk composition
- Moving Modified Partial Least Square Calibration models for milk analysis from one to another NIR portable instrument.

Figure
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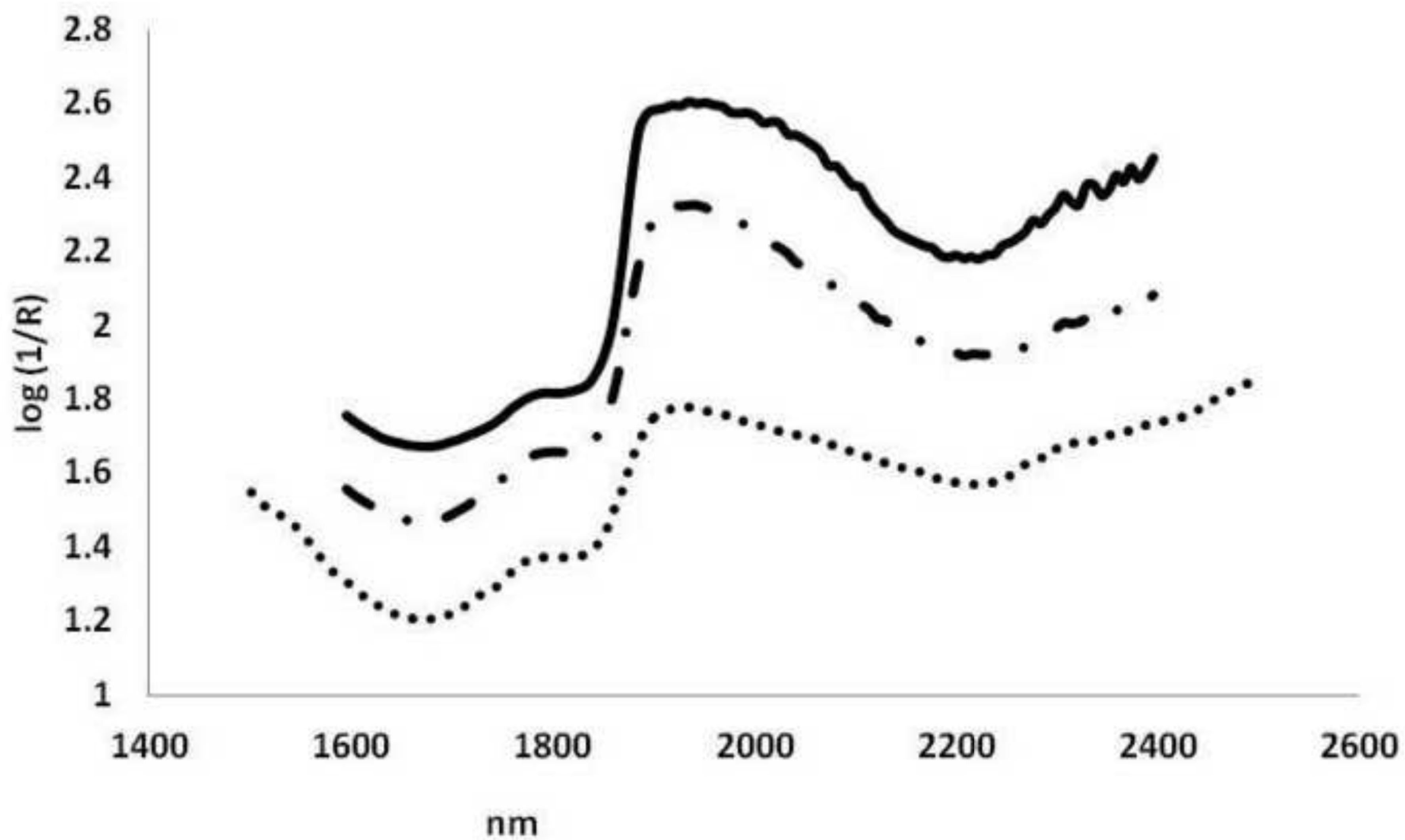

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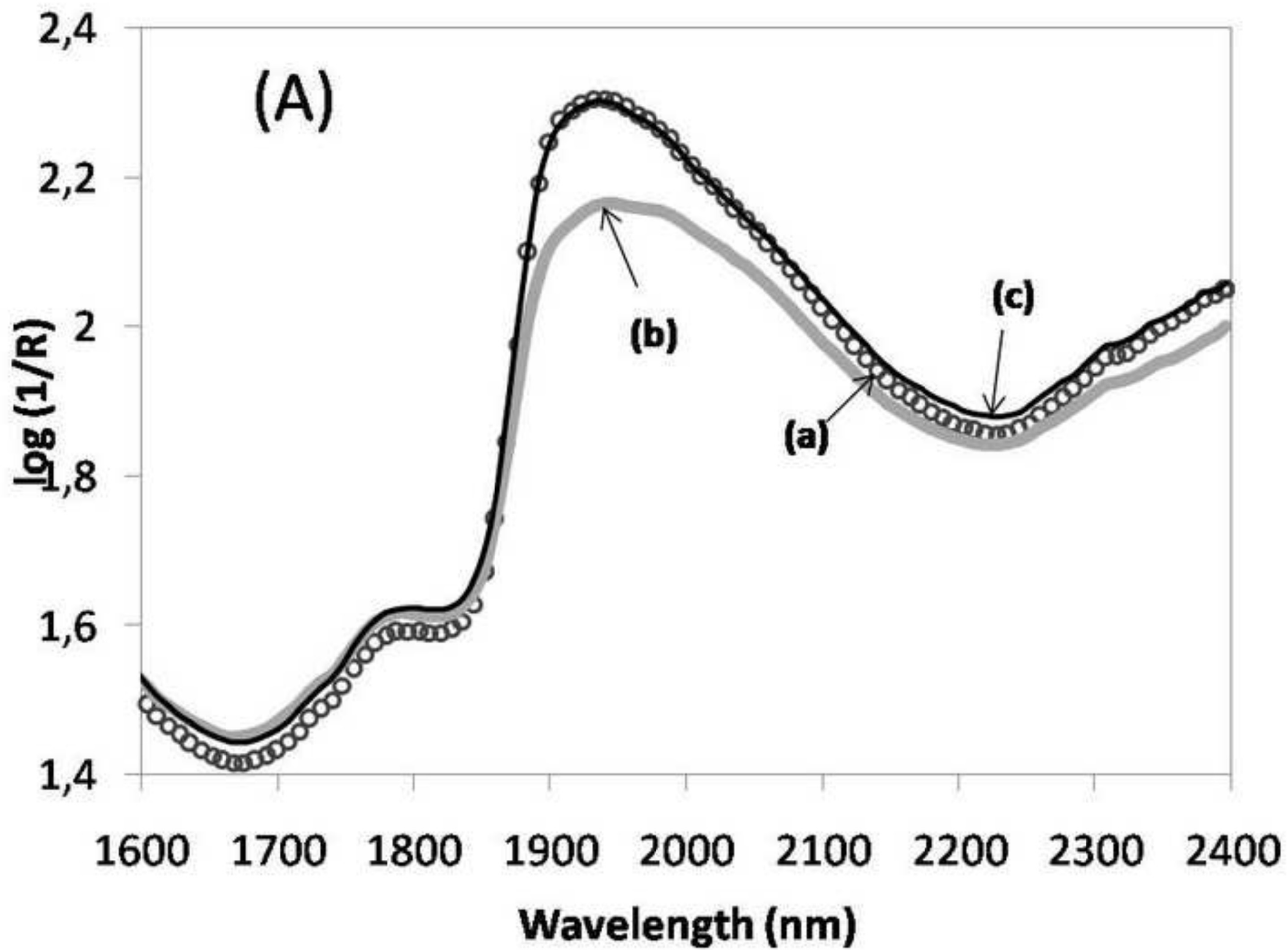

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